Recent advances in mass spectrometry-based spatially resolved molecular imaging of drug disposition and metabolomics

Running title: Mass spectrometry-based imaging of drug disposition

Yu Chen\textsuperscript{a,b,c}, Ying Liu\textsuperscript{a,b}, Ximei Li\textsuperscript{a,b}, Yan He\textsuperscript{a,b}, Weiwei Li\textsuperscript{a,b}, Ying Peng\textsuperscript{d}, Jiang Zheng\textsuperscript{a,d,*}

\textsuperscript{a}State Key Laboratory of Functions and Applications of Medicinal Plants, Key Laboratory of Pharmaceutics of Guizhou Province, Guizhou Medical University, Guiyang, Guizhou 550025, P. R. China;

\textsuperscript{b}School of Basic Medicine, School of Pharmacy, Guizhou Medical University, Guiyang, Guizhou 550025, P. R. China;

\textsuperscript{c}Division of Pain Management, The Affiliated Hospital of Guizhou Medical University, Guiyang, Guizhou 550004, P. R. China.

\textsuperscript{d}Wuya College of Innovation, Shenyang Pharmaceutical University, Shenyang, Liaoning 110016, P. R. China;

*Corresponding author

Jiang Zheng, Ph.D.

\textsuperscript{a}State Key Laboratory of Functions and Applications of Medicinal Plants, Key Laboratory of Pharmaceutics of Guizhou Province, Guizhou Medical University, Guiyang, Guizhou 550025, P. R. China;
dWuya College of Innovation, Shenyang Pharmaceutical University, Shenyang, Liaoning 110016, P. R. China.

E-mail: zhengneu@yahoo.com

Tel: +86-24-23986361

Fax: +86-24-23986510

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## Non-standard abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>PYCR2</td>
<td>Pyrroline-5-carboxylate reductase 2</td>
</tr>
<tr>
<td>GLS</td>
<td>Glutaminase</td>
</tr>
<tr>
<td>UPase1</td>
<td>Uridine phosphorylase 1</td>
</tr>
<tr>
<td>HDC</td>
<td>Histidine decarboxylase</td>
</tr>
<tr>
<td>ODC</td>
<td>Ornithine decarboxylase</td>
</tr>
<tr>
<td>ESCC</td>
<td>Esophageal squamous cell carcinoma</td>
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<tr>
<td>MIBI</td>
<td>Multiplexed ion beam imaging</td>
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<tr>
<td>ErbB-2</td>
<td>Human epidermal growth factor receptor 2</td>
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<tr>
<td>HMLE</td>
<td>Human mammary epithelial</td>
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<tr>
<td>SEAM</td>
<td>Single nuclear metabolomics</td>
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## Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
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<tbody>
<tr>
<td>MSI</td>
<td>Mass spectrometric imaging</td>
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<tr>
<td>MALDI</td>
<td>Matrix-assisted laser desorption ionization</td>
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<td>SIMS</td>
<td>Secondary ion mass spectrometry</td>
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<tr>
<td>DESI</td>
<td>Desorption electrospray ionization</td>
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<tr>
<td>LAESI</td>
<td>Laser ablation electrospray ionization</td>
</tr>
<tr>
<td>LA-ICP</td>
<td>Laser ablation inductively coupled plasma</td>
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<tr>
<td>IHC</td>
<td>Immunohistochemical</td>
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<tr>
<td>MRI</td>
<td>Magnetic resonance imaging</td>
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<tr>
<td>PET</td>
<td>Positron Emission Tomography</td>
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<tr>
<td>CT</td>
<td>Computed Tomography</td>
</tr>
<tr>
<td>LC-MS/MS</td>
<td>Liquid Chromatography-Tandem Mass Spectrometry</td>
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<tr>
<td>HPLC</td>
<td>High Performance Liquid Chromatography</td>
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<td>Abbreviation</td>
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<td>--------------</td>
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<tr>
<td>$m/z$</td>
<td>Mass-to-charge ratio</td>
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<td>2D</td>
<td>Two dimensional</td>
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<tr>
<td>TOF</td>
<td>Time-of-flight</td>
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<tr>
<td>QTRAP</td>
<td>Quadrupole ion trap</td>
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<tr>
<td>FTICR</td>
<td>Fourier-transform ion cyclotron resonance</td>
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<tr>
<td>3D</td>
<td>Three dimensional</td>
</tr>
<tr>
<td>NanoSIMS</td>
<td>Nano secondary ion mass spectrometry</td>
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<tr>
<td>AFADESI</td>
<td>Airflow-assisted desorption electrospray ionization</td>
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<tr>
<td>SDS</td>
<td>Sodium dodecyl sulfate</td>
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<td>PAGE</td>
<td>Polyacrylamide gel electrophoresis</td>
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<tr>
<td>CNS</td>
<td>Central nervous system</td>
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<td>HIV</td>
<td>Human immunodeficiency virus</td>
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<tr>
<td>FASN</td>
<td>Fatty acid synthase</td>
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<tr>
<td>GSH</td>
<td>Glutathione</td>
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<tr>
<td>Glu</td>
<td>Glutamate</td>
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<td>Gln</td>
<td>Glutamine</td>
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<tr>
<td>IMC</td>
<td>Imaging mass cytometry</td>
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<tr>
<td>COVID-19</td>
<td>Coronavirus disease 2019</td>
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<tr>
<td>IL-1β</td>
<td>Interleukin-1 beta</td>
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Abstract

Mass spectrometric imaging (MSI) is a non-targeted, tag-free, high-throughput, and highly responsive analytical approach. The highly accurate molecular visualization detection technology enables to provide qualitative and quantitative analyses of biological tissues or cells scanned by mass spectrometry in situ, extracting known and unknown multiple compounds, and simultaneously assessing relative contents of targeting molecules by monitoring their molecular ions, and pinpointing the spatial locations of those molecules distributed. Five mass spectrometric imaging techniques and their characteristics are introduced in the review, including matrix-assisted laser desorption ionization (MALDI) mass spectrometry, secondary ion mass spectrometry (SIMS), desorption electrospray ionization (DESI) mass spectrometry, laser ablation electrospray ionization (LAESI) mass spectrometry, and laser ablation inductively coupled plasma (LA-ICP) mass spectrometry. The mass spectrometry-based techniques provide the possibility for spatial metabolomics with the capability of high throughput and precision detection. The approaches have been widely employed to spatially image not only metabolome of endogenous amino acids, peptides, proteins, neurotransmitters, and lipids, but also the disposition of exogenous chemicals, such as pharmaceutical agents, environmental pollutants, toxicants, natural products, and heavy metals. The techniques also provide us with spatial distribution imaging of analytes in single cells, tissue microregions, organs, and whole animals.
Keywords: mass spectrometry, spatial distribution, drug disposition, metabolomics, secondary ion mass spectrometric, matrix-assisted laser desorption ionization mass spectrometric, desorption electrospray ionization mass spectrometric, laser ablation electrospray ionization, and laser ablation inductively coupled plasma.
**Significance statement**

The review article includes an overview of five commonly used mass spectrometers for spatial imaging and describes the advantages and disadvantages of each. Examples of the technology applications cover drug disposition, diseases, and omics. Technical aspects of relative and absolute quantification by mass spectrometric imaging and challenges for future new applications are discussed as well. The reviewed knowledge anticipates to benefit the development of new drugs and to better understand biochemical processes related to physiology and diseases.
1. Introduction

The main imaging techniques traditionally used are autoradiography, fluorescent imaging, immunohistochemical (IHC) staining, and magnetic resonance imaging (MRI). Autoradiography images whole bodies or organ systems of experimental animals. This method provides information about radioactivity localization in histological preparations at cellular level but without chemical structural identity. This technique, although sensitive, requires tedious and costly radiolabeling and organic synthesis (Solon et al., 2010; Solon, 2015). Additionally, operators to perform experiments could face harmful effects, due to exposure to radioactivity. Fluorescence often involves the detection of analytes labeled with fluorescent probes, but with challenging to simultaneously provide high resolution, speed, large volume, and good biocompatibility in a single imaging technique (Hobson and Aaron, 2022). Neither of the two can distinguish drugs from their metabolites, and they are expensive to use and have long experimental cycles. Immunohistochemical staining requires reagents specific to the targets and a low-throughput procedure (Schwamborn and Caprioli, 2010). Visualization of antibody-antigen pairs can do by optical or fluorescent imaging. MRI is an in vivo visualizing technique used for clinical diagnosis, mainly for soft tissue imaging, without ionizing radiation (Chabanova et al., 2014). Other imaging methods, such as positron emission tomography/computed tomography (PET/CT), provide little structural information using a wide range of molecular probes and metabolic parameters requiring a radiotracer (Gao et al., 2022). These imaging tools lack spatial information or lack specificity. For example, further
analysis after tissue homogenization using high-performance liquid chromatography (HPLC) or HPLC-MS/MS (tandem mass spectrometry) can easily lead to an inability to distinguish the spatial distribution of parent drugs from their metabolites (Prideaux and Stoeckli, 2012). It is also necessary to know the target molecules of interest in advance and to label the molecules with probes, such as radioisotopes, fluorescence, chemiluminescence, and other tracers. These techniques often are time-consuming, which severely drags research progresses.

MSI is a powerful label-free approach that simultaneously offers multiplexed images through acquiring hundreds of molecules and the capability to map those molecules in a sophisticated system with high flexibility and sub-cellular space analysis in technical terms (Buchberger et al., 2018a; Unsihuay et al., 2021a). MSI can provide a spatial distributional approach lacking in traditional LC-MS/MS methods and determine chemical specificity for endogenous and exogenous substances with good discrimination and selectivity for rapid screening and evaluating.

2. Mass spectrometric imaging

2.1. Principles

MSI was initially proposed by Caprioli and coworkers in 1997 (Caprioli et al., 1997) with the workflow as shown in Figure 1. Samples need to be processed properly (e.g., tissue sections are embedded in paraffin after formalin treatment or frozen at optimal cutting temperature). The areas of the selection are simultaneously scanned by use of high-energy ion beams or lasers. Biological specimens are
rasterized by label-free ionization using an ion detector to produce charged material for mass spectrometric analysis. A mass spectrometric map generates for each virtual raster pattern position. Mass analyzers can determine the mass-to-charge ratio ($m/z$) and measure intensity of ions on sample surfaces. Mass spectrometry software is used to perform the analysis, and a heat map of ion density can be generated for each $m/z$ value detected to visualize its spatial localization and relative intensity. A two-dimensional spatial dispersion of multiple molecules or ions on the sample surface builds a map. In this model, a three-dimensional spatial distribution of analytes can be generated from the two-dimensional distributed maps of the successive slices of continuously processed samples (Ye et al., 2012; Qin et al., 2018; Buchberger et al., 2018b), enabling to establish of qualitative and quantitative maps accompanied by localization functions (He et al., 2018).

2.2. Ionization techniques

The selection of ionization method is closely related to MSI’s spatial resolution and signal intensity. Several ionization techniques are commonly used: matrix-assisted laser desorption ionization (MALDI) mass spectrometry, secondary ion mass spectrometry (SIMS), desorption electrospray ionization (DESI) mass spectrometry, laser ablation electrospray ionization (LAESI) mass spectrometry, and laser ablation inductively coupled plasma (LA-ICP) mass spectrometry.

2.2.1. MALDI-MSI

MALDI involves mixing a solution of analyte molecules with a resolution of
matrix molecules which are then evaporated and dried on a MALDI target plate to form co-crystals of matrix molecules and analytes. When irradiating the crystal with laser light, the matrix crystals sublime as the matrix molecules absorb energy from the radiation and cause rapid heat production. The mass analysis measures ions' mass-to-charge ratio and ionic intensity at pixel points on the sample surface by expanding the matrix and analytes. In combination with the mass spectrometric imaging software, the signal intensity and location of an ion on the surface of the sample can be determined, and the map is constructed by establishing the 2D distribution of complementary molecules or ions on the surface of the sample (Cornett et al., 2007; Flatley et al., 2014).

MALDI-MSI is a tagless, innovative, and nascent technology that generates a two dimensional map of analytes in histopathologic tissue samples. This technique has the main advantage of determining spatial distribution of hundreds of analytes in a single imaging run without requiring markers or prior knowledge (Schnackenberg et al., 2022a). MALDI-MSI technique can record the lateral diffusion of many biomolecules concurrently, but the limited ionization hinders its sensibility. To provide high efficiency and mass precision, the most common mass analyzers for MALDI interface consist of TOF, QTRAP, Orbitrap, and FTICR (Cornett et al., 2008; Rompp et al., 2010; Alexandrov, 2012).

It is currently possible for MALDI-MSI to image spatial dispersion of biomolecules and xenobiotics in cell samples (Bien et al., 2021), spheroids (3D cultures consisting of cell polymers generated from single cell types or cell mixtures)
(Ahlf et al., 2014; Johnson et al., 2020), organ tissue sections (Goodwin, 2012a), whole body sectioning (Saigusa et al., 2019) in the form of formalin frozen and paraffin embedded tissue sections (Moginger et al., 2020). Sample preparation process mainly consists of four parts: tissue sectioning, matrix overlying, mass spectrometry analyzing, and image processing. Tissues or organs are usually snap-frozen at the point of collection with liquid nitrogen or dry-ice chilled isopentane. Freshly frozen tissues are sliced 5-15 μm thick, followed by drying to stabilize tissue samples (Goodwin, 2012b; Sun et al., 2019). Some tissues need fixing with formalin solution. The resulting tissues are dehydrated with a serial dilution of ethanol or other organic solvents and embedded in paraffin for sectioning. Appropriate matrixes, such as cyano-4-hydroxycinnamic acid, sinapinic acid, and dihydroxybenzoic acid (Jang and Kim, 2018), are sprayed onto the resultant slices which are sequentially subjected to mass spectrometric analysis. The workflow is illustrated in Figure 2 (Zhu et al., 2021a).

2.2.2. SIMS-MSI

SIMS uses a single beam of primaries injected onto the sample interface to perform measurements under a high vacuum condition, which causes the atoms on the surface to be electrostatic. The resulting secondary ions are sputtered and desorbed on the material's texture, followed by analysis using mass spectrometry and imaging by magnetic and electric fields to accurately measure the signals on the surface to be explored. The primary ion beam's energy is usually high so that the sputtering
technique breaks covalent bonds by analyzing removed samples analytes. During the
detection, many atomic and molecular debris obtained are characterized by an ion
beam sputtering the surface components within each volumetric element, a small part
of which is ionizing ionized? as either positive or otherwise negative ions. SIMS is,
therefore, generally suitable for the analysis of inorganic analytes as well as that of
substances which are chemically bonded at the surface. The mass analyzer is also
used for elemental and inorganic analysis as well as the detection of small molecules.
SIMS has the advantages of high lateral resolutions in the micron or submicron range
(Crecelius et al., 2014). The analyzers can be TOF-SIMS (Van Nuffel and Brunelle,
2022), FTICR-SIMS, and nanoSIMS (Mayali, 2020).

The procedure for tissue sectioning is the same as that for MALDI mass
spectrometric analysis. Both require frozen sample sectioning, and tissue samples for
SIMS can be analyzed directly without additional processing.

2.2.3. DESI-MSI

MALDI and SIMS mass spectrometry techniques generally require a vacuum
environment and are inconvenient to use. For this reason, Cooks’ group introduced
DESI (Takats et al., 2004) and developed it for atmospheric pressure mass
spectrometric analysis. DESI is an environmental ionization technology that requires
no substrates or specimen preparation. The basic principle includes a droplet-carrying
mechanism that impinges an atomized solvent droplet on the surface of tissue sections
to directly desorb and ionize analytes for qualitative and quantitative detection,
followed by sputtering into the gas phase and desolvation of the charged droplet by nitrogen purging and forming gas-phase ions which enter the mass spectrometric interface for analysis. DESI technique is generally considered a combination of electrospray ionization and desorption. The approach is widely used for atmospheric pressure mass spectrometric imaging. The analyzer produces mass spectra similar to ESI, mainly displaying the analyte's single or multi-charged molecular ions (Cooks et al., 2006; Beneito-Camba et al., 2020).

Solvents play a crucial role in the quality and efficiency of DESI mass spectrometry. This instrument can extract information about the distributions of different compounds on the sample surface optimized by changing the solvent systems (Eberlin et al., 2010). The distance and angle between the DESI nebulizer, the specimen surface, and the mass spectrometry entrance affects the resolution and signal intensity of imaging. In recent years, several novel techniques have been developed, such as DESI-MSI combined with ion flow separation for rapid qualification of analytes (Pierson et al., 2020a). Ion mobility spectroscopy has been merged into MSI. The relatively new technique allows to separate isomers with the same molecular weight, which has been employed for imaging particular isomers, e.g. lipids and lipopeptides (McCann et al., 2021).

Airflow assisted desorption electrosorption mass spectrometry imaging (AFADESI-MSI) improves the sensitivity of DESI-MSI detection by introducing a high-rate airflow into the ion source to strengthen the in situ collection to increase the sampling of liquid droplets. The modified DESI-MSI system is equipped with tissue
homogenization to optimize the spray solvent for analyte detection and a high-quality resolution orbital mass spectrometer with custom-developed highly discriminative imaging software MassImager. The system improves the sensitivity, specificity, and feasibility and is suitable for identifying functional molecules and tissue architecture.

Sample preparation is similar to that for MALDI-MSI analysis. Again, DESI-MSI does not require a matrix coated on sample's surface, reducing the possibility of analytes leaving the domain before analysis (Kertesz et al., 2008). Following the collection and rapid tissue freezing, sections are placed on slides and stored in a dry environment pending tests.

2.2.4. LAESI-MSI

LAESI is an innovated technology with high throughput ability and distinctive potentials for non-targeted analysis and spatial location in intact plant samples without the need of extraction or extensive specimen preparation (Kulkarni et al., 2018a). The technique uses a medium infrared laser beam to stimulate the water molecules in the samples, and the target area absorbs laser beam energy, resulting in the evaporation of the water molecules. When the energy density exceeds a critical value, a small fraction of the sample is sputtered, intercepted at right angles by the conical spray current, and ablated into gas-phase ions, which then enter the mass spectrum analyzer for group spectrometric analysis, enabling recording units of pixels at its origin. LAESI eschews the need for a remarkably flat surface, high-precision sample preparation, and matrix as a solvent. Due to these properties, it might be
good choice for spatially resolved food analysis (Nielen and van Beek, 2014). The practice of sample analysis by LAESI is similar to that of DESI. However, models are unnecessary to be dried, and plant samples should not be sectioned before analysis.

### 2.2.5. LA-ICP-MSI

LA-ICP technique is used for elemental analyses of solid materials and tissues. Liquid samples are first atomized in a sample introduction system to produce a fine aerosol and transfer it into the argon plasma applied. The high-heat plasma atomizes and electrically ionizes samples, producing ions pulled through the interfacial area and into a group of electrostatic mirrors called ion optical. The ion optomechanics focuses and guides the ion bunch into the four-pole mass spectrometer. The massive analyzer segregates the ions depending on their mass-to-charge ion ratio (m/z) and measures them on the detection unit (Wilschefski and Baxter, 2019). LA-ICP can detect and quantify proteins bound with metal-labeled antibodies (Sussulini et al., 2017). Electrophoretic protein segregation (PAGE or SDS) allows to analyze metal-containing proteins to be visualized in 2D gels. LA-ICP with multiple element ability enables to accurately measure and visualize metals in tissues with high spatial resolution, flexibility, quantitative capability, and excellent repeatability (Weiskirchen et al., 2019). In the analysis of biological samples, LA-ICP combined with TOF allows to identify proteins labeled with a trace of elements.

The five techniques have their own characteristics as listed in Table 1 and Figure 3. With a high resolution, SIMS-MSI can offer 3D imaging without requiring matrixes.
However, the method is only suitable for detecting small molecules with low sensitivity, making it more difficult to provide quantitative data. DESI-MSI requires neither complex sample pretreatment nor matrixes, facilitating the expansion of the analysis to liquid and gas samples. However, the technique has the weakness of relatively poor resolution and low signal susceptibility to external environmental interference. LAESI-MSI sample preparation is simple, compassionate, and matrix-free but requires aqueous and relatively stable samples and shows poor resolution. MALDI-MSI technique provides high resolution and is suitable for all biological tissue samples with no molecular weight limitation. However, sample presentation and matrices can disturb the detection of analytes (Porta et al., 2018). LA-ICP-MSI offers elemental imaging, trace metal detection, and quantification of metal-labeled antibodies, but isobaric interference lacks matrix-matched standards for quantification (Spruill et al., 2022a).

3. Spatially resolved imaging and applications

3.1. Drug disposition

Various methods for measuring and visualizing medication uptake, distribution, and excretion have been explored for MSI. Nilsson and coworkers studied propranolol, metoprolol, and atenolol absorption in small intestines of rats using a quantitative MALDI-MSI technique (Nilsson et al., 2017). They identified the sites for intestinal absorption of the three b-blockers. Chen and coworkers used MALDI-TOF-MSI to visualize detailed distribution of five CNS drugs in the brain of mice after
intraperitoneal administration with these agents (Chen et al., 2020). Gruner and coworkers evaluated erlotinib, an inhibitor of epidermal growth factor receptor for the therapy of pancreatic ductal adenocarcinoma, and found that the agent was spatially distributed in healthy pancreas and unhealthy pancreas of mice using MALDI-MSI (Gruner et al., 2016). Cesca and coworkers employed MALDI-MSI to examine the potentiating effect of bevacizumab on paclitaxel-mediated anticancer activity and succeeded in establishing 3D distribution of the two antitumor agents in tumor sections (Cesca et al., 2016).

The information about fixation-related effects on MSI of metabolomics and drug disposition is limited. Dannhorn and coworkers assessed the changes in endogenous metabolome and xenobiotics in rat liver and kidney tissues using DESI- and MALDI-MSI. DESI does not require a complicated pretreatment process. The mass spectra of small molecules, such as amino acids, organic amines, lipids, and fatty acids, can be directly detected on freshly frozen sections. However, the technique does not work well for formalin-fixed paraffin-embedded samples, due to low sensitivity. MALDI-based technique has the ability to analyze exogenous substances and metabolites as well as endogenous lipids and proteins with high sensitivity. Little difference in sensitivity of detection is obtained between the analysis of formalin-fixed samples and that of freshly-frozen samples. No significant changes in the sensitivity to detect diphenhydramine, dextromethorphan, and terfenadine distributed in liver and kidney were observed in formalin fixed and freshly frozen organs obtained from animals after the treatment of the antihistamine agents (Dannhorn et al., 2022). However, freshly
frozen tissues are recommended to be used for imaging the distribution of low abundant analytes, such as metabolites of losartan, terfenadine, and fexofenadine (Schnackenberg et al., 2022b).

Seneviratne and coworkers successfully imaged the distribution of tenofovir and its active metabolites in colorectal biopsies in healthy volunteers, using MALDI-MSI (Seneviratne et al., 2018). Later, they examined the spatial distribution of emtricitabine, tenofovir, efavirenz, and rilpivirine, alone with some endogenous biomolecules, in the heart, liver, spleen, kidney, and brain in mice treated with the four anti-HIV agents and succeeded in detecting these antiretroviral agents in the organs analyzed (Seneviratne et al., 2020). Ntshangase and coworkers used MALDI-MSI combined with LC-MS/MS to assess the pharmacokinetics and spatial distribution of efavirenz, tenofovir, and emtricitabine in the brain in rats after intraperitoneal injection of the three agents. Tenofovir was mainly located in the cortex, emtricitabine was mainly distributed in the thalamus, corpus callosum and hypothalamus, and efavirenz was found in the brain (Ntshangase et al., 2019).

3.2. Diseases

3.2.1. Tumors

The metabolic re-programming of carcinoma cells, a new hallmark of cancer, occurs during tumor growth and progression. Cancer cells independently alter their throughput by various metabolic pathways to meet the elevated bioenergetic and biological synthetic demands and to reduce the oxidative stress required for cancer
cell multiplication and survival. Besides the known cytological and molecular resistance mechanisms, other factors can influence the distribution and concentration of chemotherapeutic agents in tumors (Dey et al., 2021). The metabolism of the tumor immune micron environment by off-tumor cells, including endothelium, fiber, and immune cells, should be considered in targeting cancer therapy (Martinez-Reyes and Chandel, 2021; Stine et al., 2022).

Guenther and co-workers found higher levels of fatty acids and phospholipids in patients with breast tumors using DESI-MSI technique. The metabolomic work attempted to define the association of endogenous substances, such as estrogens, progesterones, fatty acids, and phospholipids, with the tumor's gradings (Guenther et al., 2015a). Abliz's team identified abnormal expressions of six enzymes, including pyrroline-5-carboxylate reductase 2 (PYCR2), glutaminase (GLS), uridine phosphorylase 1 (UPase1), histidine decarboxylase (HDC), fatty acid synthase (FASN), and ornithine decarboxylase (ODC) in 256 patients with esophageal squamous cell carcinoma (ESCC) by use of AFADESI-MSI (Figure 4) (Sun et al., 2019a). The spatial integration of distinguished enzymes and the corresponding downstream metabolites would facilitate the understanding of tumor metabolism and discovery of new metabolic pathways.

Recently, MSI technologies combined with MALDI, SIMS, and DESI have been quickly applied in animal and clinical studies of breast, kidney, ovarian, prostate, colon, brain, lung, thyroid, skin, pancreatic, and esophageal cancers. Similarly, rapid development has been achieved in metabolomic (e.g., lipids, fatty acids, amino acids,
and glucose) imaging of various tumors (Cho et al., 2017; Inglese et al., 2017; Jirasko et al., 2017; Zhang et al., 2017; Bluestein et al., 2018; Paine et al., 2019; Zang et al., 2021). The above techniques may be beneficial for the development of biomarkers and metabolic pathway discovery. High-resolution mass spectrometric imaging is an invaluable tool for metabolomic analysis of cancer tissues, recording the differential production of biomolecules, and specifying the spatial distribution within the samples, which facilitates precise treatment of tumors.

3.2.2. Other diseases

MSI has been used to identify disease-related pathological features, diagnose diseases, and evaluate the effectiveness of intervention. The technique is employed in neurological diseases, such as Alzheimer's disease (Cruz-Alonso et al., 2019), Parkinson's disease (Shariatgorji et al., 2014), Huntington's disease (Hunter et al., 2018), multiple sclerosis (Maccarrone et al., 2017), amyotrophic lateral sclerosis (Hanrieder et al., 2013), and frontotemporal dementia (Agrawal et al., 2022). Kaya and coworkers observed co-localized lipids such as ceramide, sulfatides, phosphatidylinositol, and lysophosphatidylcholine with plaque-associated Aβ subtypes in the hippocampal region of Alzheimer's disease transgenic mice, using MALDI-MSI (Kaya et al., 2017). These findings suggested a definite link between amyloid accumulation and lipid metabolism alterations in response to oxidative stress, inflammation, demyelination, and the death of cells. MSI technique is as well used in the study of rheumatic immune diseases, and images of articular cartilage, synovium,
and bone have been successfully constructed. This facilitates the better understanding of joint destruction and further characterizing and diagnosing osteoarthritis, rheumatoid arthritis, and osteoporosis (Rocha et al., 2017) as well as developing predictive biomarkers for these rheumatic immune diseases. The technology is also employed in kidney and aging-related diseases, providing detailed diagnostic information by recording ion imaging of the spatial distribution of endogenous molecules and proteins as well as exogenous substances in specimens and by distinguishing the differential distribution of those small and large molecules in humans and experimental animals (Abbas et al., 2019; Rossiter et al., 2022).

3.3. Omics

3.3.1. Metabolome

Grove and coworkers investigated mechanistic action of the hepatotoxicity of amodiaquine, a well-known antimalarial agent, using MALDI-MSI. Depletion of glutathione, along with the production of amodiaquine-derived GSH conjugate, was visualized in the central lobular region of the liver in rats given amodiaquine. Additionally, accumulated parent amodiaquine was observed in the periportal area relative to the centrilobular area (Grove et al., 2019). The spatial imaging findings provided histologic details for the metabolic activation of amodiaquine responsible for the reported idiosyncratic hepatotoxicity. bis-Choline tetra thiomolybdate is a therapeutic agent for various cancers, Wilson's disease, and multiple sclerosis. Foster and co-workers determined the distribution of the agent in different organs and tissues,
such as suprarenal gland, liver, spleen, kidney, brain, and testis using LA-ICP mass spectrometry, and they found the accumulation of molybdenum in these organs of animals receiving a high dose of the metal agent (Foster et al., 2022). The team was also defined the correlation between the accumulation of molybdenum and its toxicities.

Resistance to clinic-associated bacterial specimens, including enterobacteriaceae, non-ferme strains, and other bacteria, has become an issue of concern. Florio and co-workers successfully developed several methods for rapid detection of metabolites of antibiotics associated with antimicrobial resistance, using a combined MALDI-TOF mass spectrometry approach. In-depth studies of the most common methods include the mapping of particular distribution of β-lactamase by visualizing hydrolytic products of β-lactam antibiotics (Florio et al., 2020).

MSI is also a powerful tool for new drug discovery. This technique allows us to directly visualize the distribution of drug candidates as well as their metabolites, which are necessary for quick decisions in drug development. Using the AFADESI-MSI technique, He and co-workers constructed spatially resolved imaging of N²-(4-hydroxy benzyl)-adenosine, an anti-insomnia drug candidate, in the brain of rats, which allowed them to image the spatial distribution of the drug candidate, facilitating the understanding of pharmacologic action of this particular candidate (He et al., 2015a).

Metabolomics often refers to the collective study of endogenous small biomolecules within cells, biofluids, tissues or organisms and of their interactions
within a biological system and has been being developed rapidly in recent decades and shown remarkable promise in multiple fields, such as chemistry, life sciences, and clinical medicine. In 2020, Nature Methods named "Tools for Metabolomics" as one of the methods to look forward to (Singh, 2020). Spatial multi-omics, including spatial metabolomics, was named by Nature in 2022 as one of the top seven technologies of the year, with a greater emphasis on spatial distribution in the xyz axis. Integration of mass spectrometry imaging technology with metabolomics is called spatially resolved metabolomics. The advantage is mainly to study the space distributional characteristics and spatiotemporal dynamics of biomolecules in tissue sections to visualize the overall metabolome in situ (Fox and Schroeder, 2020a).

In 2015, He and coworkers combined AFADESI-MSI technology with metabolomics and proposed a new methodology for mass spectrometric imaging with metabolomics (He et al., 2015b). In the same year, Guenther and coworkers employed DESI-MSI technique for the detection of endogenous substances to diagnose breast cancer (Guenther et al., 2015b). These laid the groundwork for the application of spatially resolved metabolomics.

In 2019, Abliz and coworkers established an entire body spatial resolution imaging approach to monitoring the distribution of YZG-331 and YZG-330, two isomeric sedative and hypnotic drug candidates, and their metabolites. The same technique was employed to image the changes in the allocation of neurotransmitters in the brain of rats administered individual epimers. They succeeded in defining the correlation between the distribution of each epimer in the brain vs. stomach with their efficacy. Additionally, they found the changes in levels of glutamate (Glu) and glutamine (Gln) in the cerebral brain of rats (Figure 5) (Luo et al., 2019) were
consistent with the effectiveness of the two isomeric drug candidates. The team continued to mine the distribution of other drugs regarding spatial metabolomic dynamics and related pharmacologic properties (Zhang et al., 2020). Additionally, the team used AFADESI and MALDI to image the location of endogenous sugars, amino acids, nucleotides, fatty acids, lipids, vitamins, peptides, and metal ions associated with diabetic nephropathy across renal tissues in a rat model of diabetic nephropathy. And visualization of the improvement of metabolic disorders was observe in the animal model after 12-week administration of oral astragaloside IV (Wang et al., 2021).

Recently, He and coworkers determined the 3D distributional changes in metabolome resulting from the exposure to exogenous substances, such as drug candidates, toxicants, and herbal medicines. Those endogenous substances monitored included amino acids and their metabolites, lipids, neurotransmitters, and biomolecules associated with energy metabolism. Furthermore, the team succeeded in defining the correlation of those xenobiotics with multiple endogenous substances from the perspective of specific locations (Liu et al., 2020; Pang et al., 2021; Cong et al., 2022; Gao et al., 2022; Jiang et al., 2022; Liu et al., 2022). 3D-MSI has been used to visualize the distribution of pharmaceutical agents and enabled to establish the relationship between the structural heterogeneity of the microenvironment and the distribution of drugs within the tissues (Giordano et al., 2016, Vos et al., 2019). It is anticipating that more 3D studies will be performed with the development of sophisticated and advanced computational systems and bioinformatic methods.

3.3.2. Proteome

Spatial mapping of the distribution of proteins has become possible by using
mass spectrometry. The method of mass spectrometry lies on incorporating ionizable metal attached to specific antibodies. Angelo’s team used a multiplexed ion beam imaging (MIBI) technique, a secondary ion mass spectrometry method for imaging antibodies labeled with isotopically pure elemental metals. They succeeded in spatially localizing estrogen receptor, progesterone receptor, e-calmodulin, Ki67 protein, vimentin, actin, keratin, and receptor tyrosine-protein kinase erbB-2 stained with metal-coupled antibodies to analyze paraffin-embedded sections of human breast tumor tissues (Angelo et al., 2014). Giesen and coworkers employed imaging mass cytometry (IMC) technology combined with a high-resolution laser ablation system and CyTOF-a time-of-flight LA-ICP-MS instrument mass cytometry to visualize human mammary epithelial (HMLE) cells and human breast cancer samples by simultaneously imaging 32 proteins and their modifications at a cellular resolution of 1.0 μm (Giesen et al., 2014).

Rendeiro and coworkers analyzed 36 proteins expressed in cultured primary pneumocytes and lung tissues obtained from patients with COVID-19 and constructed spatial distribution of these proteins by mass spectrometric cytometry with high Query String imaging. The success provided comprehensive and spatial pictures of the human lung responses to the viral infection from macro to single-cell levels. Specifically, infiltrated monocytes with the expression of IL-1β were found in the lung of patients with early COVID-19 infection, while high degrees of inflammation, macrophage infiltration, complement activation, and fibrosis were observed in the lungs of individuals with COVID-19 late stages. The findings not only provided
structural, immunological, and clinical insights into lung pathology but also plotted a landscape of lung pathologic changes (Rendeiro et al., 2021).

Hamidi and coworkers used MALDI-TOF mass spectrometry to distinguish bacteria *Brucella abortus* from *Brucella melitensis*. Additionally, significant protein mass signals were successfully identified for ribosomal and structural proteins for each vaccine and virulent strain represented by the corresponding biomarker peaks (Hamidi et al., 2022). Mass spectrometry-based spatial proteomics can determine the nature of proteoform and subcellular localization, which enables to better understand the complexity of protein morphology, along with the significance of exploring unknown functions (Lundberg and Borner, 2019).

### 3.3.3. Single cell

Single cell analysis is a single cell-based study of genomics, transcriptomics, proteomics, metabolomics and cell–cell interactions (Wang and Bodovitz, 2010; Merouane et al., 2015). Single-cell spatial imaging may be used to detect the co-localization of the distribution of specific molecular species, such as particular lipids and proteins, and to define the association with morphological characteristics of tissue sections, both of which are crucial for molecular pathology and cancer treatment (Rappez et al., 2021a). Small-size cells require high resolution for imaging. Due to analyte delocalization and degradation, matrix crystal size, laser focusing limitation, and detector sensitivity are hurdles for spatial resolution (Scupakova et al., 2020). The latest SpaceM, an open-source system for *in situ* single-cell metabolomics based on
MALDI and light microscopy, can detect over 100 molecules per hour in >1,000 individual cells with fluorescent readouts and morpho spatial features. The developed technique is currently applied to identify the metabolic state within genetic cell lines, such as differentiated human hepatocytes (Rappez et al., 2021b).

Yuan and coworkers developed a spatial single nuclear metabolomics (SEAM) approach, a multiscale spatial resolution platform combining wet experiments with computational algorithms, to characterize metabolic intra- or inter-cellular features. This technique allowed them to visualize the tissue architecture of cultured primary hepatic cells and to distinguish hepatocytes, Kupffer cells, and endothelial cells of mouse liver sections by monitoring various biomolecules responsible for those individual types of cells. Furthermore, the technique enabled them to image the elevated transmembrane transport of amino acids in human fibrotic liver tissue (Yuan et al., 2021).

Single-cell imaging is a growing technology in biomedical sciences in three aspects, including single-cell microscopy combined with microfluidics, mass spectrometric imaging for subcellular compound localization, and imaging mass cytometry. The operations of the approach not only require centralized identification and maintenance of cell culture conditions while being able to receive high-resolution imaging for extended periods of time, but also necessitate the development of new and advanced equipment (Skylaki et al., 2016). The challenges of single-cell imaging include the complexity of data acquired and the requirement of more advanced computational analysis and bioinformatic methods for data processing, due to their
multivariate nature (Pomerantz et al., 2019).

4. Conclusion and perspectives

MSI technique provides label-free and in situ imaging with high coverage and wide detection ranges. This relatively new analytical approach has been widely employed in biomedical research and clinical practice. The imaging with spatial information is achieved by mass spectrometry-based detection of various endogenous and exogenous substances. The endogenous substances include amino acids, peptides, proteins, neurotransmitters, lipids, and other biomolecules (Goodwin et al., 2008; Luan et al., 2019; Denti et al., 2020; Nachtigall et al., 2020). Varieties of exogenous chemicals, such as pharmaceutical agents (their metabolites), environmental pollutants, toxicants, natural products, heavy metals, and others, have been analyzed for molecular imaging. The most important and advantageous features of the technology is that the mass spectrometry-based molecular imaging allows us to establish spatial distribution and accumulation of the detected molecules in a variety of organisms. Those mass analyzers include MALDI, SIMS, DESI, LAESI, LA-ICP, and their combinations. These applications can certainly benefit life sciences in the future. However, these techniques are currently limited by the low spatial resolution of DESI and even MALDI. The instrument conditions, sample preparation, and data processing all need to be improved (Heeren et al., 2009). In particular, increases in detection sensitivity and resolution will be the immediate efforts in the future (Hou et al., 2022).
Quantitation is an important issue for any analytical approaches. Unfortunately, limited iteration of absolute quantitative MSI is available so far. During the sample processing phase, an authentic standard of known concentration is often deposited onto an adjacent untreated control sample that is being processed and analyzed simultaneously, allowing the estimation of absolute quantification. This method was applied to determine the abundance of tiotropium bromide in rat lung tissue sections (Goodwin, 2012c). Relative quantification relies on internal standards or selected endogenous molecules in samples to obtain relative changes in the concentration of other molecules in different samples. This approach is commonly used for the non-targeted analysis of systematic phenotypic changes under different conditions (Unsihuay et al., 2021c).

The rapid development of metabolomics provides a large room for the improvement of spatial metabolomics imaging. This would allow us to visualize the metabolome in situ to picture the spatial distribution of characteristic biomolecules and to construct a more accurate foundation for biochemical processes in the whole living species (Fox and Schroeder, 2020b). Tumor heterogeneity and its microenvironment are biologically responsible for tumor staging, grading, and classification. Spatial metabolomics provides a novel approach not only precisely to diagnose tumors by identifying tissue metabolic heterogeneity and cellular microenvironment but also to provide new strategies for targeting treatment of cancers. Molecular imaging also offers a powerful tool for the determination of the spatial distribution of pharmaceutical agents and their metabolites, dramatically
facilitating new drug discovery (Miao et al., 2018; Vitale et al., 2021; Wang et al., 2022; Zhang et al., 2022).

The disposition of medicinal herbs is more complicated than that of a pure pharmaceutical agent. Little progress has been achieved in the application of MSI to spatial mapping distributed chemical components in animals after exposure to traditional Chinese medicines. MSI technique is certainly able to provide spatial qualitative and quantitative identities of multiple chemical components distributed in tissues, organs, and whole body in animals given herbal medicines. Such technique would facilitate the understanding of mechanisms of therapeutic and toxic actions of medicinal herbs with increasing consumption.

Single-cell imaging, 3D targeted imaging, and quantitative metabolomics would play an increasingly important role in future standardization and translation for clinical practice (Chen and Abliz, 2017; Blutke et al., 2020; Zhou et al., 2020).

The merge of multidimensional mass spectrometry, biochip, artificial intelligence, and other technologies would promote the development of life sciences and other related fields (Li et al., 2022).
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Authorship Contributions

Wrote or contributed to the writing of the manuscript: Yu Chen, Ying Liu, Ximei Li, Yan He, Weiwei Li, Ying Peng, Jiang Zheng.
References


Chabanova E, Larsen L, Logager VB, Moller JM and Thomsen HS (2014) [Use of magnetic resonance imaging]. *Ugeskr Laeger* 176:50-54.


investigate the hepatotoxicity mechanisms of component D of Polygonum multiflorum Thunb. *J Ethnopharmacol* 298:115630.


Kulkarni P, Wilschut RA, Verhoeven K, van der Putten WH and Garbeva P (2018a) LAESI mass spectrometry imaging as a tool to differentiate the root metabolome of native and range-expanding


Moore KL, Chen Y, van de Meene A, Hughes L, Liu W, Geraki T, Mosselmans F, McGrath SP,


Pierson EE, Midey AJ, Forrest WP, Shah V, Olivos HJ, Shrestha B, Teller R, Forster S, Bensussan A


Schnackenberg LK, Thorn DA, Barnette D and Jones EE (2022a) MALDI imaging mass spectrometry: an emerging tool in neurology. *Metab Brain Dis* 37:105-121.

Schnackenberg LK, Thorn DA, Barnette D and Jones EE (2022b) MALDI imaging mass spectrometry: an emerging tool in neurology. *Metab Brain Dis* 37:105-121.


Spruill ML, Maletic-Savatic M, Martin H, Li F and Liu X (2022a) Spatial analysis of drug absorption, distribution, metabolism, and toxicology using mass spectrometry imaging. *Biochem Pharmacol*


**Footnotes**

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Legends

**Figure 1.** Schematic workflow of mass spectrometry imaging (MSI). Created with BioRender.com

**Figure 2.** Schematic diagram of preparation of various specimens, inducing cells, spheroids, organs, whole-body sections, and paraffin-embedded tissue sections for MSI constructions. Reprinted with permission from (Zhu et al., 2021a). Copyright © 2022 Zhu, Xu, Peng and Wu. (https://pubmed.ncbi.nlm.nih.gov/35186891).

**Figure 3.** Comparison of several MSI ionization techniques. Reprinted with permission from (Spruill et al., 2022b). Copyright © 2022. Published by Elsevier Inc. The figure was created with the addition of LAESI and using BioRender.com. (https://www.sciencedirect.com/science/article/abs/pii/S0006295222001745).

**Figure 4.** Six abnormally expressed metabolic enzymes in esophageal squamous cell carcinoma by AFADESI-MSI. (A-F) Quantification of immunohistochemistry signals of the six enzymes, including PYCR2, GLS, Upase1, HDC, ODC, and FASN in cancer, paired epithelium, and muscle tissues obtained from 256 ESCC patients. Reprinted with permission from (Sun et al., 2019b). Copyright © 2019 the author(s). Published by PNAS. (https://pubmed.ncbi.nlm.nih.gov/30559182).

**Figure 5.** Mass spectrometry imaging of stomach and brain in vehicle or YZG-331/YZG-330 treated rats. (A) Distribution of glutamic acid (Glu, m/z 148.0604) and glutamine (Gln, m/z 147.0764); (B) Relative intensity of Glu and Gln in the stomach.
and brain; (C) Relative intensity ratio of Glu vs. Gln in the stomach and brain.

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Table1. Characteristics of different mass spectrometry images.
<table>
<thead>
<tr>
<th>Ion source</th>
<th>Scanning beam</th>
<th>Pressure regime</th>
<th>Mass range (Da)</th>
<th>Spatial resolution (mm)</th>
<th>Application</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Dynamic SIMS</strong></td>
<td>Primary charged particles</td>
<td>Vacuum/L</td>
<td>1-300</td>
<td>0.03–0.5</td>
<td>Elements and small atomic clusters</td>
<td>(Watrous and Dorrestein, 2011).</td>
</tr>
<tr>
<td><strong>Static SIMS</strong></td>
<td>Primary charged particles</td>
<td>Vacuum/L</td>
<td>100-1,500</td>
<td>0.5–50</td>
<td>Low molecular mass compounds (&lt; 1,000 Da)</td>
<td>(Debois et al., 2008; Kertesz and Van Berkel, 2008; Becker et al., 2010).</td>
</tr>
<tr>
<td><strong>LDI</strong></td>
<td>UV laser</td>
<td>Vacuum, IP, or AP</td>
<td>100-5,000</td>
<td>1–500</td>
<td>Monitoring of molecular species (containing a chromophore compatible with the laser used)</td>
<td>(Holscher et al., 2009; Bartels and Svatos, 2015a; Dong et al., 2016).</td>
</tr>
<tr>
<td><strong>MALDI</strong></td>
<td>UV or IR laser</td>
<td>Vacuum, IP, or AP</td>
<td>300-50,000</td>
<td>1-100</td>
<td>All kinds of biological samples (Small molecules, lipids, peptides, and proteins)</td>
<td>(Korte et al., 2015; Shariatgorji et al., 2015; Bai et al., 2016; Sturtevant et al., 2016; Zhu et al., 2021b).</td>
</tr>
<tr>
<td>Technique</td>
<td>Spray Type</td>
<td>Pressure</td>
<td>Mass Range</td>
<td>Sample Type</td>
<td>References</td>
<td></td>
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<tr>
<td>DESI</td>
<td>Charged</td>
<td>AP</td>
<td>100-5,000</td>
<td>Small, lipids, and proteins (&lt; 2000 Da)</td>
<td>(Tillner et al., 2017; Pierson et al., 2020b).</td>
<td></td>
</tr>
<tr>
<td>Nano-DESI</td>
<td>Liquid</td>
<td>AP</td>
<td>100-10,000</td>
<td>Same as DESI</td>
<td>(Moore et al., 2014; Unsihuay et al., 2021b).</td>
<td></td>
</tr>
<tr>
<td>LAESI</td>
<td>IR laser</td>
<td>AP</td>
<td>1-66,000</td>
<td>Small and large molecules</td>
<td>(Bartels and Svatos, 2015b; Kulkarni et al., 2018b; Bartels and Svatos, 2022).</td>
<td></td>
</tr>
<tr>
<td>LA-ICP</td>
<td>UV laser</td>
<td>AP</td>
<td>7-250</td>
<td>Elements, trace metal detection and quantification</td>
<td>(Becker et al., 2010; Chen et al., 2022).</td>
<td></td>
</tr>
</tbody>
</table>

LP, low pressure; IP, intermediate pressure; AP, atmosphere pressure.
Figure 1